# LINC01638 regulates miR-128/PDK1 to promotes cell proliferation and drug resistance in ovarian cancer

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**Research Article**

**Keywords:** LINC01638, ovarian cancer, PDK1, miR-128-3p, cisplatin resistance

**Posted Date:** April 5th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1509617/v1

# Abstract

This study aims to explore the functional mechanism of LINC01638 in ovarian cancer (OC). The OC cell proliferation, viability and colony formation were respectively measured using CCK-8, MTT and colony formation assay. The cell cycle and apoptosis were both assessed using flow cytometry. Online bioinformatics tools were performed to predict the downstream gene of LINC01638. RNA pull down experiment and luciferase reporter assay were processed to verify the predictions. The expression of LINC01638, miR-128-3p and PDK1 in OC tissues and cells were detected using qRT-PCR and ISH analysis. The expressions of cisplatin-related proteins were determined by western blot. OC mice model was constructed to carry out in vivo experiment. LINC01638 was highly expressed in OC tissues and cells, and its high expression was related to poor OC prognosis. LINC01638 knockdown inhibited cell proliferation, colony formation and cisplatin resistance, promoted cell apoptosis and induced cell cycle arrest in OC cells. LINC01638 knockdown suppressed tumor growth, proliferation and cisplatin resistance and enhanced apoptosis in vivo. LINC01638 directly targeted miR-128-3p/PDK1 in OC cells. LINC01638 affected OC cell proliferation, survival and cisplatin resistance via combing miR-128-3p and promoting PDK1 expression.


# Introduction

In female reproductive system, ovarian cancer (OC) is the most lethal cancer [1]. The incidence of OC is second only to cervical cancer and endometrial cancer, ranking third in female malignant tumors [2, 3]. Worldwide, a total of 300,000 new OC cases and 200,000 deaths from OC were reported in 2018 [4]. In China, there were 52,100 new cases and 22,500 deaths of OC in 2016 [5]. The onset of OC is concealed. And when the patients are with ascites, abdominal distension and anorexia are found, most of the lesions have metastasis, and most of them are in advanced clinical stage [6].

OC is mainly treated by cytoreductive surgery and combined chemotherapy with cisplatin and paclitaxel [7]. Cisplatin is widely employed in the clinical treatment of OC and used as an antineoplastic drug. Although the initial sensitivity rate of OC patients to cisplatin-based combination chemotherapy can reach more than 80%, cisplatin-based resistance is very easy to occur [8]. Most patients eventually relapse and die from chemotherapy resistance [9]. Therefore, reducing the recurrence rate and reversing the chemoresistance of OC, especially the mechanism and reversal pathway of cisplatin resistance, have become an important research topic.

RNA that is longer than 200 nucleotides and lacks the ability to encode proteins is called long noncoding RNA (lncRNAs) [10]. About 76% of human genome transcription produces lncRNAs, which are located in the cytoplasm and nucleus [11]. With the development of high-throughput sequencing and other advanced research technologies, the biological functions of lncRNAs are becoming more and more clear [12]. Increasing evidence suggests that lncRNA plays a significant role in many different cellular processes, including cell migration, growth, invasion, apoptosis and differentiation. However, the biological function and molecular mechanism of lncRNA in human diseases (including cancer) remain a

mystery [13]. LINC01638 was reported as an oncogene and associated with cell proliferation, migration and invasion in several cancers, such as breast cancer [14], colorectal adenocarcinoma [15] and prostate carcinoma [16]. However, the functional role of LINC01638 played in OC was still unclear.

In this study, we firstly found that LINC01638 was over-expressed in OC tissues and cell lines. And, we confirmed that the silence of LINC01638 inhibited OC cell proliferation and cisplatin resistance and promoted cell apoptosis *in vivo* and *in vitro*. LINC01638 knockdown also arrested OC cell cycle. In addition, we demonstrated that LINC01638 affected cell proliferation, cell cycle, apoptosis and cisplatin resistance of OC by regulating miR-128-3p/3-Phosphoinositide-dependent protein kinase-1 (PDK1). Our study proved that LINC01638 played an important role in OC and might be a novel strategy in OC research.

# Materials And Methods

### Patients and tissues

OC tissues samples (n = 63) and matched peritumor normal (non-tumor, defined as tissues surrounding the tumor) tissues (n = 63) were harvested from OC patients from May 2015 to September 2017 in the First Affiliated Hospital of Shangqiu Medical College in Henan Province. The samples used in this study were immersed in liquid nitrogen for 10 min and then stored at -80 ℃. This study was approved by the First Affiliated Hospital of Shangqiu Medical College in Henan Province ethics committee (No.2019021). The written informed consent forms were signed and submitted by all of these OC patients. The OC tissues were subsequently divided into LINC01638 low- and high- expressed groups based on the expressions of LINC01638, and the median of LINC01638 expression level was seemed as the cut-off value. The period from surgery to death was described as overall survival and the overall survival of these OC patients was recorded. The relationship between LINC01683 expressions and OC clinical pathology (Ages, FIGO stage, Grade, Distant metastasis and Tumor size) was analyzed.

### In situ hybridization (ISH) analysis

A hybridization solution (Sangon Biotech, Shanghai, China) was used to pre-hybridize OC tissues sides at 37℃ for 2 h. A total of 10 picomoles of digoxigenin-labeled detection probes (Boster Bioengineering, Wuhan, China) complementary to LINC01683 were added and cultured at 37℃ overnight. After washing, a mouse monoclonal antibody to digoxigenin (ab420, Abcam, Cambridge, MA, USA) was used to carry out an immunologic reaction, whereafter alkaline phosphatase-conjugated streptavidin dilution (Boster) was added to determine streptavidin dilution probes. After drying, slides were observed and photographed under a microscope (Leica, German).

### Cell culture

OC cell lines, including SKOV3, CAOV3, OVCAR4 and OVCAR3, and normal cell line IOSE80 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). The Dulbecco's

Modified Eagle Media (DEME) medium, containing 10% fetal bovine serum (FBS, Invitrogen Carlsbad, CA, USA), was applied to culture the cell lines in this study. The cells were then cultured at 37 ℃ in an incubator with 5% $CO_2$.

## Cell transfection

The LINC01638 knocked down plasmid/control plasmids (pLKO.1-shLINC01638/pLKO.1), LINC01638 overexpressed plasmid/control plasmid (pBabe-puro-LINC01638/pBabe-puro), microRNAs-128-3p (miR-128-3p) mimics/negative control (NC) mimics (miR-128 mimics/NC mimics) and miR-128 inhibitor/NC inhibitor were purchased from GenePharma (Shanghai, China). Human OC cell lines OVCAR3 and SKOV3 were transfected with 30 nM of pLKO.1-shLINC01638/pLKO.1, pBabe-puro-LINC01638/pBabe-puro, miR-128 mimics/NC mimics or miR-128 inhibitor/NC. And HEK 293T cells were transfected pLKO.1-shLINC01638/pLKO.1, pBabe-puro-LINC01638/pBabe-puro or miR-128 mimics/NC mimics in the 12-well culture plates using Lipofectamine 2000 (Invitrogen, USA). After transfected for 48 h, all of the cells were collected and used for the following experiments.

## Drug treatment

After the OC cell lines OVCAR3 and SKOV3 were transfected with pLKO.1-shLINC01638/pLKO.1, pBabe-puro-LINC01638/pBabe-puro or miR-128 mimics/NC mimics, the cells were treated with cisplatin (2 μg/mL). Dimethyl Sulfoxide (DMSO, Invitrogen) served as the drug of control group.

## CCK-8 assay

Stably transfected OC cells in this study were incubated in 96-well plates ($5 \times 10^3$ cells/well) with DMEM medium (100 μL, containing 10% FBS) and were cultured at 37°C, 5% $CO_2$. After cultured for 1, 2, 3 and 4 days, CCK-8 kit (10 μL, Dojindo, Kumamoto, Japan) was added for 4 h at 37 °C. Plates were subsequently putted in a microplate reader (Biotek, USA) to detect the optical density (OD) value at 450 nm wavelength.

## MTT assay

After transfection, OC cell lines were treated with 0 nM, 0.25 nM, 1 nM, 4 nM, 16 nM, 64 nM, 256 nM and 1024 μM cisplatin for 24 h. At the end of treatment, the MTT assay was used to detect the cell viability. The OC cells in this study were incubated in 96-well plates ($5 \times 10^3$ cells/well) with DMEM medium (100 μL, containing 10% FBS) and were cultured at 37°C, 5% CO2. After cultured for 48 h, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltet razolium bromide (MTT kits, Sigma-Aldrich, St. Louis, USA) was added into each well for 4 h at 37 °C. The MTT solution was discarded, and then 150 μL DMSO were added into the plates. After mixing, the OD value of 570nM was detected using microplate reader (Biotek). A GraphPad Prism software 6.0 (San Diego, CA, USA) was performed to calculated the IC50 values.

## Colony formation assay

Transfected OC cells (400 per group) from each treatment were seeded in 12-well plates for 14 days. After that, crystal violet was employed to stain the OC cell lines. An invert microscope was used to count the number of stained cells. (Olympics, Tokyo, Japan).

### Flow cytometry

After the OC cell lines transfected for 48 h, the cells were collected. For cell cycle analysis, the cells were fixed and permeabilized using 75% cold ethanol, washed using PBS and resuspended using propidium iodide buffer (Sigma). Cell cycle of all this cells were measured by processing flow cytometry (BD Biosciences, NJ, USA) was processed for detecting cell cycle. For apoptosis assays, after harvesting cells, these human OC cells were stained by fluorescein isothiocyanate-conjugated Annexin V (Nanjing KeyGen Biotech, Nanjing, China) and propidium iodide (Nanjing KeyGen Biotech). Flow cytometry was then operated to assess cell apoptosis.

### Xenograft tumor model assay

Female Bal/c nude mice (6-8 weeks; n = 6/group) were purchased from Beijing HFK Bioscience Co. Ltd. (Beijing, China) and raising in sterility environments with approval by the First Affiliated Hospital of Shangqiu Medical College in Henan Province. Human OC cell lines OVCAR3, which were transfected with pLKO.1-shLINC01638 or pLKO.1, were hypodermic injected into mice with a density of $1\times10^7$ cells. When the volume of the tumors grew to 200 $mm^3$, cisplatin (10 mg/kg) was injected into these nude mice, vehicle served as control group. The mice were divided into 4 groups: pLKO.1 + vehicle, pLKO.1 + Cisplatin, pLKO.1-shLINC01638 + vehicle and pLKO.1-shLINC01638+Cisplatin. The tumor volume was measured and recorded weekly for 35 days after cisplatin injection, Tumor volume ($mm^3$) = $0.5\times L\times W^2$, the length was represented as L and the width was represented as W. The mice were anesthetized by intraperitoneal injection of 10% chloral hydrate with the density of 400 mg/kg, and then euthanized by air embolization after 5 weeks. The tumors were removed for tumor weight examination after death confirmation by cardiac arrest and pupil enlargement. And, tumor tissues were harvested for subsequent experiment. Animal experiments were proceeded in accordance with relevant guidelines and regulations of the First Affiliated Hospital of Shangqiu Medical College in Henan Province.

### Ki-67 expression

The paraffin was applied to fix and embed tumor tissues. The tissues were into pieces (5 μm) and placed in glass slides. Then the glass slides were rehydrated and paraffinized via xylene and graded alcohols, respectively. The target retrieval solution was used to immerse the slides for 30 min in a water bath. The endogenous peroxidase was blocked in $H_2O_2$ (3%) for 15 min, and the nonspecific bindings was buried in goat serum for 50 min. Then, the slides were stained with Ki-67 primary antibodies were applied to stain the slides, following by secondary antibody polymer HRP was employed to stain the slides. 3,3'-diaminobenzidine (DAB) was subsequently used to stain the slices stained and methyl green was used to counterstained them. Images were taken under a light microscope.

## TUNEL staining

The apoptosis of mice tumor tissues was monitored using an in situ cell death detection kit (Roche, Basel, Switzerland). In brief, the tissue sections were blocked using $H_2O_2$ (3%) for 5 min and then defined using TdT labeling reaction mix for 1 h. The experiment was processed at 37°C. The sections were stained by DAB for 15 min and observed under a light microscope.

## RNA pull down assay

The target miRNA and gene of LINC01638 were predicted using miRDB and Targetscan 7.1, respectively. RNA pull down assay was carried out in human OC cell line OVCAR3 with the silence of LINC01638 or overexpression of LINC01638 to validate whether miR-128-3p could combine with LINC01638. In brief, the biotinylated pLKO.1-shLINC01638/pLKO.1 or pBabe-puro-LINC01638/pBabe-puro probes were inoculated in streptavidin-coupled magnetic beads for 48 h. And qRT-PCR was performed to detect the purified RNA complexes expression. This experiment was processed under 25°C.

## Luciferase reporter gene assay

Luciferase reporter vectors, containg PDK1-WT and PDK1-Mut, were constructed. HEK293T cells were transfected with miR-128 mimics/NC, pLKO.1-shLINC01638/pLKO.1 or pBabe-puro-LINC01638/pBabe-puro and then respectively cotransfected with the above luciferase reporter vectors. After 48 h transfection, the double-luciferase assay system (Promega, USA) was proceeded to assess the cells luciferase activity.

## RNA extraction and qRT-PCR

LINC01638, miR-128-3p and PDK1 expressions in OC tissues and cells was measured by qRT-PCR. In brief, TRIzol (Sigma-Aldrich) was applied to harvest the total RNA in tissues and cells. The cDNA was synthesized by the reverse transcription reaction of RNA. PrimeScript™ RT reagent kit (Takara, Japan) was used to conduct the reverse transcription reaction according to the instructions. The expression levels of LINC01638, miR-128-3p and PDK1 were then detected by qRT-PCR using SYBR Premix Ex Taq™ kit (Takara). ABI-7900 thermal cycling instrument used in this study was provided by Applied Biosystems, USA. The relative expressions of LINC01638, miR-128-3p and PDK1 were analyzed by $2^{-\Delta\Delta CT}$ method. GAPDH was served as internal control. The primer sequences were as follows: LINC01638 forward primers: 5'-AATACATCAGCACTGTTGCCTTT-3', reverse primers: 5'-CTCCATACATACATCTCCAAAAAGT-3'; miR-128-3p forward primers: 5'-GGGTCACAGTGAACCGGT-3', reverse primers: 5'-TCCTCCTCTCCTCTCCTCTC-3'; PDK1 forward primers: 5'-CCCCTCAGCTTGCAGAT-3', reverse primers: 5'-TGCTCCACACAGTCATTCA-3'; GAPDH forward primers: 5'-AAGGTCGGAGTCAACGGA-3', reverse primers: 5'-TTAAAAGCAGCCCTGGTGA-3'.

## Western blot

Briefly, lysis buffer was used to lyse cells used in the present study, and then total proteins in cells were obtained. BCA protein assay kit, provided by BioVision, USA, was applied to measure the protein concentration of each sample. The sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate these proteins, and then these separated proteins were electrically transferred to the PVDF membrane. The PVDF membrane was blocked by 5% skimmed milk powder, and then primary antibodies, provided by Abcam, was added to incubate the membrane overnight at 4°C and the secondary antibody was added for 2 h at room temperature. The primary antibodies used in this study was as follow: anti-P-glycoprotein (anti-p-gp) (1:1000, ab242104), anti-poly (ADP)-ribose polymerases (anti-PARP, 1:1000, ab191217), anti-cleaved-PARP (anti-c-PARP, 1:1000, ab32064), anti-PDK1 (1:1000, ab110025) and anti-β-actin (1:1000, ab8227). The target bands were respectively reflected and evaluated by ECL and Quantity One software (Bio-Rad, Hercules, CA, USA). β-actin was served as internal control.

### Statistical analysis

Data from this study were analyzed by SPSS 22.0 (IBM, USA) and indicated as mean ± SD of at least three independent experiments. To compare the differences among groups, Student's t-test, one-way ANOVA or two-way ANOVA were employed. Pearson's $\chi 2$ tests were performed to analyze the relationship between LINC01638 expression and OC clinical pathology, including Ages, FIGO stage, Grade, Distant metastasis and Tumor size. Kaplan-Meier survival analysis was carried out for analysis of survival rate. The correlations among LINC01638, miR-128-3p and PDK1 expressions in OC tissues were determined using Spearman's correlation analysis. The log-rank test was applied to calculate P-value, and $P<0.05$ presented statistically significant.

# Results

### LINC01638 was highly expressed in OC cell lines and tissues and related to OC prognosis

We clinically collected 63 pairs of OC tumor tissue samples and non-tumor tissue samples, and qRT-PCR was used to measure the relative expression of LINC01638 in these tissue samples. Data from qRT-PCR showed that significant high expressions of LINC01638 was found in 63 OC tumor samples compared to that in non-tumor tissue samples ($P<0.001$, Figure **1A**). ISH was performed to detect the expression of LINC01638 in the tissue samples, and the result was shown as Figure **1B**. Figure **1B** also exhibited that the expression of LINC01638 in OC tissues was dramatically higher than that in non-tumor tissues.

The overall survivals of 63 OC patients were recorded and then analyzed using Kaplan-Meier survival analysis. As shown as Figure 1C, patients with high LINC01638 expression (n = 32) was associated with lower overall survival than that with low LINC01638 expression (n = 31) ($P<0.05$). The relationship between LINC01638 and OC prognosis was also assessed, and the results were summarized as Table **1**. Table 1 revealed that high LINC01638 expression was related to advanced FIGO stage ($P<0.05$) and grade ($P<0.01$), positive distant metastasis ($P<0.01$) and lager tumor size ($P<0.01$).

In addition, four human OC cell lines (SKOV3, CAOV3, OVCAR4 and OVCAR3) and normal cell line IOSE80 were obtained and subjected to qRT-PCR analysis to determine the expression levels of LINC01638. Data from qRT-PCR displayed that much LINC01638 expressions in SKOV3, CAOV3, OVCAR4 and OVCAR3 cell lines was observed compared with that in IOSE80 cell line ($P$<0.01). Among the four cell lines tested, LINC01638 expressed the highest in OVCAR3 and the lowest in SKOV3. Therefore, OVCAR3 and SKOV3 were selected for subsequent experiments.

### Knockdown LINC01638 inhibited proliferation and survival of OC cells

To verify whether knockdown of LINC01638 expression affected OC cells activities, the knockdown plasmid of LINC01638 pLKO.1-shLINC01638 or empty plasmid pLKO.1 were transfected into OCVAR3 and SKOV3 cell lines and the transfection efficiency was measured via qRT-PCR. Figure **2A** showed that, after transfection, the expressions of LINC1638 in two pLKO.1-shLINC01638 groups were both notably down-regulated compared to that in pLKO.1 groups ($P$<0.01).

The proliferative activities of OCVAR3 and SKOV3 cell lines were detected using CCK-8 assay. The significant lower OD value was observed in cells of pLKO.1-shLINC01638 groups at $4^{th}$ day compared to that of pLKO.1 groups (Figure **2B**, $P$<0.01). MTT assay was performed to measure the effects of LINC01638 down-regulation on the cisplatin resistance of OCVAR3 and SKOV3 cell line. Data from MTT assay exhibited that, the cisplatin IC50 values of pLKO.1-shLINC01638 groups in OCVAR3 and SKOV3 cell line were both significantly lower than that of pLKO.1 groups (Figure **2C**, $P$<0.01). It indicated that the knockdown of LINC01638 promoted the cisplatin sensibility of OC cells. The clonality of cells was measured using colony formation assay and the result was showed as Figure 2D. Similarly, the clonality of cells in pLKO.1-shLINC01638 groups was prominently lower than that in pLKO.1 groups (Figure **2D**, $P$<0.01). These results demonstrated that LINC01638 knockdown inhibited cell proliferation, drug resistance and cell colony formation in OC cell lines.

Next, flow cytometry was enabled to examine the effect of LINC01638 knocked down on OC cells survival. As shown as Figure **2E**, the cell apoptosis percentages (Sum of early and late apoptosis) of pLKO.1-shLINC01638 groups were markedly higher than that of pLKO.1 groups ($P$<0.01). The cell cycle was also evaluated by flow cytometry. It could be seen from Figure **2F**, in human OC cell lines OVCAR3 and SKOV3, the percentages of cells in G1 phase in pLKO.1-shLINC01638 groups were significantly higher than that in pLKO.1 groups ($P$<0.01), which suggested that LINC01638 knocked down caused OC cell cycle arrest.

### LINC01638 caused cisplatin resistance in OC *in vivo* and *in vitro*

OC cell lines OVCAR3 and SKOV3 were both transfected with LINC01638 knockdown plasmid pLKO.1-shLINC01638 or overexpression plasmid pBabe-puro-LINC01638, and subsequently treated with cisplatin. Then, the protein expressions of p-gp, PARP and c-PARP were measured using western blot.
When OVCAR3 and SKOV3 were transfected with pBabe-puro-LINC01638/pBabe-puro, both of the cell lines were divided into 4 groups: pBabe-puro + DMSO, pBabe-puro-LINC01638 + DMSO, pBabe-puro +

Cisplatin and pBabe-puro-LINC01638 + Cisplatin. Data from western blot showed that, when the expression of LINC01638 were up-regulated in OC cells, an increase of p-gp and a decrease of c-PARP were found (Figure **3A**). Furthermore, When OVCAR3 and SKOV3 were transfected with pLKO.1-shLINC01638/pLKO.1, both of the cell lines were also divided into 4 groups: pLKO.1 + DMSO, pLKO.1-shLINC01638 + DMSO, pLKO.1 + Cisplatin, pLKO.1-shLINC01638 + Cisplatin. The expressions of p-gp were significantly declined as well as the expression of c-PARP were obviously enhanced in pLKO.1-shLINC01638 groups compared to that in pLKO.1 groups (Figure **3B**, $P<0.01$). And, the supplement of cisplatin had no effect on these changes induced by LINC01638 over- or down- regulation. These results suggested that the expression of LINC01638 could affect the sensitivity of OC cells to cisplatin.

To further explore the effect of LINC01638 knockdown on OC *in vivo*, OC nude mice models were constructed. The mice were injected with OVCAR3 cells that were treated with the combined of pLKO.1-shLINC01638/pLKO.1 and cisplatin/vehicle. And then the mice were divided into four groups: pLKO.1 + vehicle, pLKO.1 + Cisplatin, pLKO.1-shLINC01638 + vehicle and pLKO.1-shLINC01638 + Cisplatin. The tumor size and weight of OC mice were significantly decreased in pLKO.1-shLINC01638 groups compared to that in pLKO.1 groups ($P<0.01$), and when further treated with cisplatin, the tumor size and weight of pLKO.1-shLINC01638 + Cisplatin group were even decreased compared to that of pLKO.1 + Cisplatin group ($P<0.05$, Figure **3C, D** and **E**). The images from Ki-67 assay exhibited that both pLKO.1-shLINC01638 transfection and cisplatin treatment could reduce the degree of Ki-67 staining, and the co-treatment of pLKO.1-shLINC01638 and cisplatin showed the lightest staining degree (Figure **3F**, $P<0.01$). Data from TUNEL assay were also showed in Figure 3F, the TUNEL staining degree was enhanced after pLKO.1-shLINC01638 transfection or cisplatin treatment, and the pLKO.1-shLINC01638 + Cisplatin group displayed the deepest staining effect ($P<0.01$). These results indicated that the knockdown of LINC01638 could inhibit OC tumor growth, proliferation and drug resistance and promote apoptosis *in vivo*.

### LINC01638 targeted miR-128-3p/PDK1 in OC

We predicted miR-128-3p was the target miRNA of LINC01638 by miRDB, and the binding sites of LINC01638 on miR-128-3p were showed as Figure **4A**. After pBabe-puro-LINC01638/pBabe-puro or pLKO.1-shLINC01638/ pLKO.1 transfection, RNA pull down experiment was performed on OVCAR3 cells, and qRT-PCR was used to detect the enrichment of miR-128-3p. As shown as Figure **4B**, the enrichment of miR-128-3p was significantly reduced when LINC01638 up-regulated, and the knockdown of LINC01638 obviously enhanced miR-128-3p enrichment ($P<0.01$). The expressions of miR-128-3p in 63 OC tissue samples were measured using qRT-PCR, and the correlation between LINC01638 and miR-128-3p was analyzed by SPSS. In OC tissue samples, the LINC01638 expression was negatively related to the miR-128-3p expression (Figure **4C**, $P<0.001$). These results revealed that LINC01638 directly targeted miR-128-3p in OC.

Next, we predicted PDK1 directly bound to miR-128-3p using Targetscan (Figure **4D**), and luciferase reporter gene assay was performed to verify the prediction. Luciferase vectors, including PDK1-WT and PDK1-Mut, were constructed to assess the luciferase activity. Data from luciferase reporter gene assay

showed that the relative luciferase activity of PDK-1-WT was evidently decreased with the miR-128-3p overexpression or LINC01638 knocked down, and obviously increased with LINC01638 overexpression (Figure **4E**, *P*<0.01). In 63 OC tumor tissues, the PDK1 expression was positively related to LINC01638 expression and negatively associated with miR-128-3p expression (Figure **4F**). In addition, miR-128-3p expression was up-regulated in OC cell lines OCVAR3 and SKOV3 by miR-128-3p mimics transfection, and then the relative protein expression of PDK1 was detected using western blot. Data from western blot exhibited that the overexpression of miR-128-3p significantly suppressed the protein expression of PDK1 (Figure **4G**, *P*<0.01). Furthermore, we up- or down- regulated the expression of LINC01638 in OC cell lines OCVAR3 and SKOV3, meanwhile, the expression level of miR-128-3p was also up- or down- regulated, and then the PDK1 expression was measured via western blot. After overexpression of LINC01638 in OCVAR3 and SKOV3 cell lines, the expression level of PDK1 prominently increased, and continued to overexpress miR-128-3p in cells, while the expression level of PDK1 dramatically decreased (Figure **4H**, *P*<0.01). After knockdown of LINC01638 in OCVAR3 and SKOV3 cell lines, the expression level of PDK1 significantly declined, and continued to knock down miR-128-3p in cells, the expression level of PDK1 obviously increased (Figure **4I**, *P*<0.01). The above results confirmed that LINC01638 could regulate the expression level of miR-128-3p, thereby affecting the expression level of target gene PDK1.

### LINC01638 affected OC cell proliferation, survival and cisplatin resistance through regulating miR-128-3p

To further validate the mechanism of LINC0168 in OC cell activity, rescue experiment was performed. The OCVAR3 was transfected with pBabe-puro-LINC01638/pBabe-puro and miR-128-3p mimics, and the cells were divided into 3 groups: pBabe-puro, pBabe-puro-LINC01638 and pBabe-puro-LINC01638 + miR-128-3p mimics. After transfection, CCK-8 assay was applied to detect the OCVAR3 cell proliferation. As shown as Figure **5A**, cell proliferation was notably enhanced after LINC01638 overexpression, while the simultaneous overexpression of miR-128-3p destroyed the promotion effect of LINC01638 on cell proliferation (*P*<0.01). Data from MTT assay showed that cisplatin IC50 value was significant decreased with LINC01638 overexpression, and the decrease was reversed with the miR-128-3p overexpression at the same time (Figure **5B**, *P*<0.01). Similarly, the colony cell number was obviously added after pBabe-puro-LINC01638 transfection, and declined after miR-128-3p mimics transfection (Figure **5C**, *P*<0.01). After the overexpression of LINC01638, the apoptosis rate and G1 percentage were decreased, while the overexpression of miR-128-3p broke the inhibition effect of LINC01638 on apoptosis and cell cycle (Figure **D** and **E**). Furthermore, the protein expressions of p-gp, PARp and c-PARP were measured through western blot. After the overexpression of LINC01638, p-gp protein significantly increased and c-PARP obviously decreased. However, after miR-128-3p overexpressing in OC cells, p-gp expression showed a decrease as well as c-PARP showed an increase (Figure **5F**). It suggested that LINC01638 led to cisplatin insensitivity mediated by miR-128-3p.

## Discussion

OC is one of the gynecological malignant tumors with the highest mortality rate [1, 2]. Because early diagnosis cannot be made without definite symptoms, the vast majority of patients with OC are

diagnosed in late stage [3]. Currently, OC is usually treated by surgery and chemotherapy combined with cisplatin and paclitaxel, as well as appropriate radiotherapy [17]. Although OC patients have short-term remission, recurrence and metastasis rates are still high. Traditional therapies are limited due to lack of in-depth understanding of drug resistance and molecular mechanisms of OC progression. In order to better understand the specific molecular mechanisms in OC, more efforts are needed to explore new diagnostic and therapeutic strategies. At present, there are few biomarker for predicting the response to chemotherapy in clinical practice. Therefore, the discovery of new biomarkers is of great significance for the early diagnosis, prognosis and reduction of drug resistance of OC.

Previous studies investigated that lncRNA as a biomarker could play a role in a variety of cancers. In the study of OC, the role of lncRNAs had gradually become a hotspot. Liang *et al*. [18] reported that lncRNA PTAR accelerated invasion-metastasis and epithelial-to-mesenchymal transition (EMT) in OC via binding miR-101-3p to modulate ZEB1 expression. Wang *et al.* [19] found that the lncRNA TP73-AS1 played a oncogenic role and promoted cell metastasis and proliferation in OC cells by regulating MMP2 and MMP9 expressions. Li *et al*. [20] reported that lncRNA TP73-AS1 modulated cell apoptosis, cell cycle and proliferation in OC cells and its high expression predicted poor prognosis. However, more studies are needed to understand the role of lncRNAs in OC.

LINC01638 was proved to be an oncogene in some cancers. Liu *et al*. [21] found that LINC01638 was over-expressed in breast cancer cells and tissues, and they also confirmed that LINC01638 could affect breast cancer cell apoptosis, proliferation and invasion *in vivo* and *in vitro*. Zhuo *et al*. [15] reported that LINC01638 was highly expressed in colorectal adenocarcinoma tissues and cells, and the knockdown of LINC0163 suppressed colorectal adenocarcinoma cancer cell proliferation through targeting RUNX2. However, the functional role of LINC01638 played in OC remains unclear. In the current study, we found that LINC01638 was highly expressed in OC tissues and cells. The LINC01368 inhibition among the four cell lines (SKOV3, CAOV3, OVCAR4 and OVCAR3) and IOSE80 is one of the limitations of this study and will be used as a follow-up study. Then, our study investigated that the high expression of LINC01638 predicted poor prognosis and lower overall survival of OC patients. The expression of LINC01638 is down-regulated in OC cell lines OCVAR3 and SKOV3. We confirmed that LINC01638 knockdown could inhibit OC cell proliferation, cisplatin resistance and colony formation after performing CCK-8 assay, MTT assay and colony formation assay, respectively. In addition, our data elucidated that the silence of LINC0638 promoted cell apoptosis and induced cell G1 phase arrest in OC cells by running flow cytometry.

gp was reported to be positively related to cisplatin resistance [22] while c-PARP was found to be negatively related to it [23]. Besides, p-gp [24] and c-PARP [25] were also found to be abnormal expressed in OC tissues and cell lines and related to the occurrence, development and cisplatin resistance of OC. We observed that LINC01638 overexpression increased p-gp expression and reduced c-PARP expression. On the other hands, LINC01638 knockdown reduced p-gp expression and increased c-PARP expression. It indicated that the overexpression of LINC01638 could lead to cisplatin insensitivity in OC cells. In addition, we illustrated that the knockdown of LINC01638 could repress OC tumor growth, proliferation

and cisplatin resistance and promote apoptosis *in vivo* through constructing OC mice models. However, due to the limitation of experimental conditions, the synergy effect of LINC01638 and cisplatin on OC has not been studied in this paper, and the synergy effect needs to be further studied.

Online bioinformatics tools were employed to predict the downstream target of LINC01638 to further explore its molecular mechanism in OC. MiR-128-3p was reported to be a tumor suppressor in hepatocellular carcinoma [26], colorectal cancer [27] and glioma [28], and could inhibit cancer cell proliferation and metastasis. We predicted and verified that LINC01638 bound with miR-128-3p by miRDB and RNA pull down assay, respectively. Previous studies confirmed that PDK1, an oncogene, was related to cell activities and highly expressed in a variety of cancers, such as lung cancer [29], breast cancer [30] and OC [31]. Targetscan and luciferase reporter gene assay were respectively performed to predict and verify that miR-128-3p targeted PDK1. Then, we showed that LINC01638 and PDK1 expressions in OC tissues and cell lines were negatively correlated with miR-128-3p expression, and LINC01638 in OC tissues and cell lines was positively related to PDK1 expression. In addition, rescue experiment were carried out and proved that overexpression of LINC01638 in OC cells increased cell proliferation, viability, colony formation and cisplatin resistance and decreased cell apoptosis and G1 phase percentage, while overexpression of miR-128-3p in OC cells eliminated the above cell activities induced by LINC01638 overexpression. It revealed that LINC01638 promoted cisplatin resistance through targeting miR-128-3p.

In conclusion, the present study demonstrated that LINC01638 was highly expressed in OC tissues and cells, and LINC01638 regulated OC cell cycle, proliferation, apoptosis and cisplatin resistance through modulating miR-128-3p/PDK1. It suggested that LINC01638 might be a expectable biomarker in OC therapy.

# Declarations


### Funding

Not applicable.

### Acknowledgements

Not applicable.


### Compliance with ethical standards

### Conflict of interest

All authors in this study declare that they have no conflict of interests.

### Ethics approval and consent to participate

The project protocol was approved by the First Affiliated Hospital of Shangqiu Medical College in Henan Province.

### Author contributions

YY and HW designed the study, performed the experiments, wrote the main manuscript and analyzed the data. Both of the authors read and approved the final manuscript.

### Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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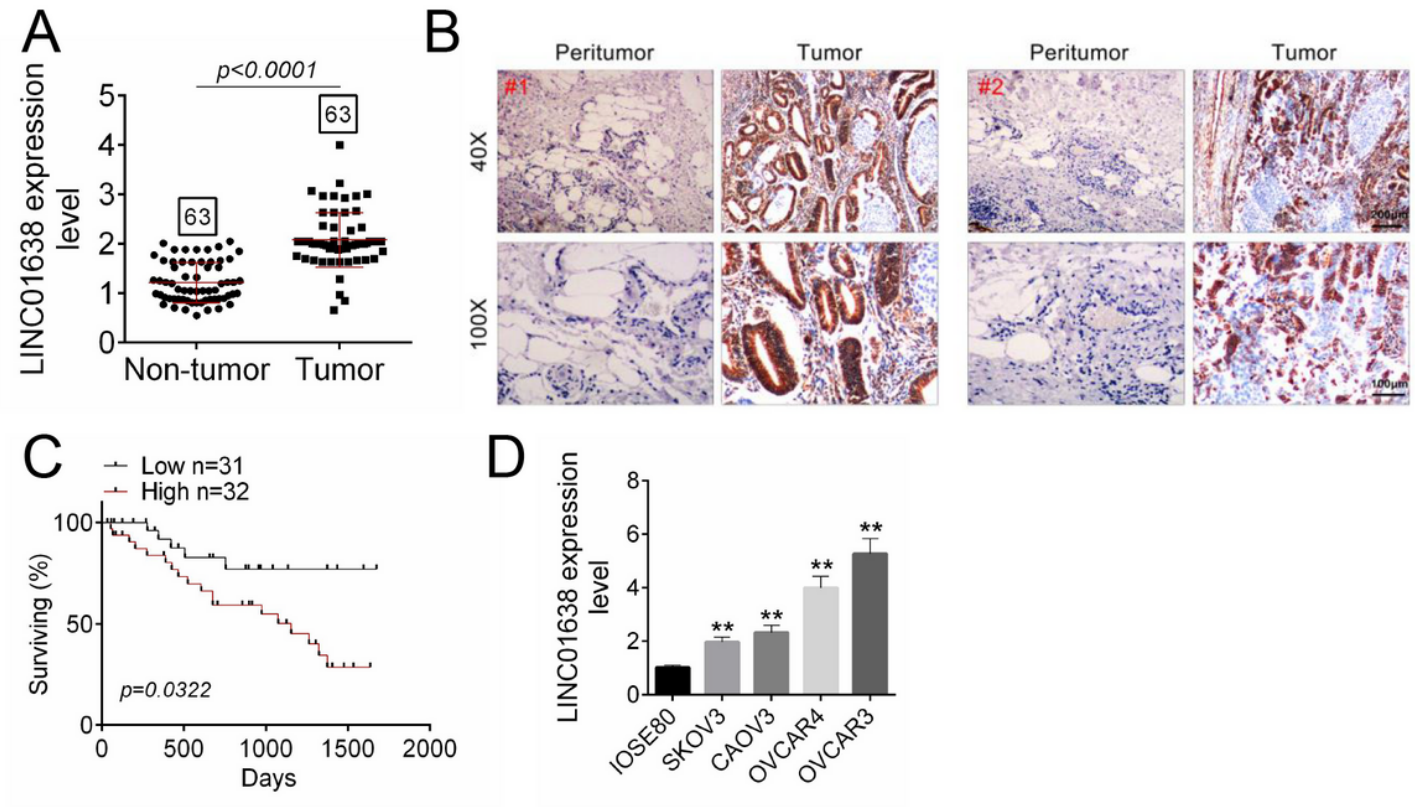
# Tables

**Table 1 Relationship between LINC01683 expression and clinical features of OC patients**

| Characteristics | Number of patients | LINC01638 Low expression (<median) | LINC01638 High expression (≥ median) | P value |
|---|---|---|---|---|
| Number | 63 | 31 | 32 | |
| Ages(years) | | | | 0.244 |
| <55 | 27 | 11 | 16 | |
| ≥55 | 36 | 20 | 16 | |
| FIGO stge | | | | 0.027 |
| I&II | 38 | 23 | 15 | |
| III&IV | 25 | 8 | 17 | |
| Grade | | | | 0.001 |
| G1 | 23 | 18 | 5 | |
| G2&G3 | 40 | 13 | 27 | |
| Distant metastasis | | | | 0.016 |
| Yes | 19 | 5 | 14 | |
| No | 44 | 26 | 18 | |
| Histotype | | | | 0.885 |
| Papillary-serous | 52 | 25 | 27 | |
| Mucinous | 3 | 2 | 1 | |
| Endometrioid | 1 | 0 | 1 | |
| Clear cell | 3 | 2 | 1 | |
| Undifferentiated | 2 | 1 | 1 | |
| Adenocarcinoma | 2 | 1 | 1 | |
| Tumor size (mm) | | | | 0.016 |
| ≤10 | 25 | 17 | 8 | |
| >10 | 38 | 14 | 24 | |

# Figures

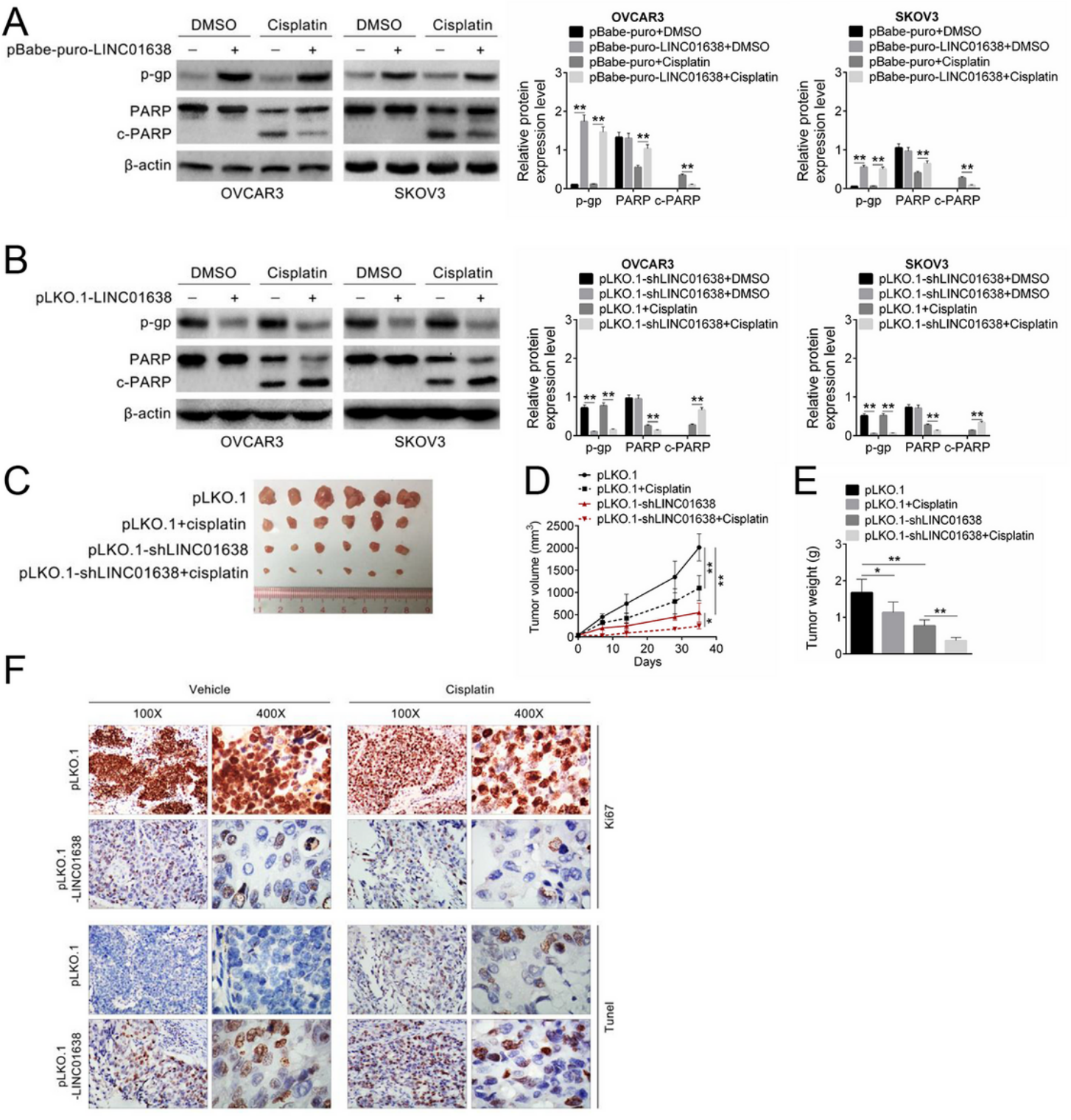



## Figure 1

**LINC01638 was up-regulated in OC.** (A) The LINC01638 expression in 63 pairs in OC tumor tissues and matched non-tumor tissues was detected using qRT-PCR. (B) The LINC01638 expression in OC tumor tissues and matched non-tumor tissues was detected using ISH. (C) The overall survivals of 63 OC patients was analyzed using Kaplan-Meier survival analysis. (D) The LINC01638 expression in OC cell lines (SKOV3, CAOV3, OVCAR4 and OVCAR3) and normal cell line IOSE80 was detected using qRT-PCR. ***P*<0.01 vs non-tumor group or IOSE80 cells.

## Figure 2

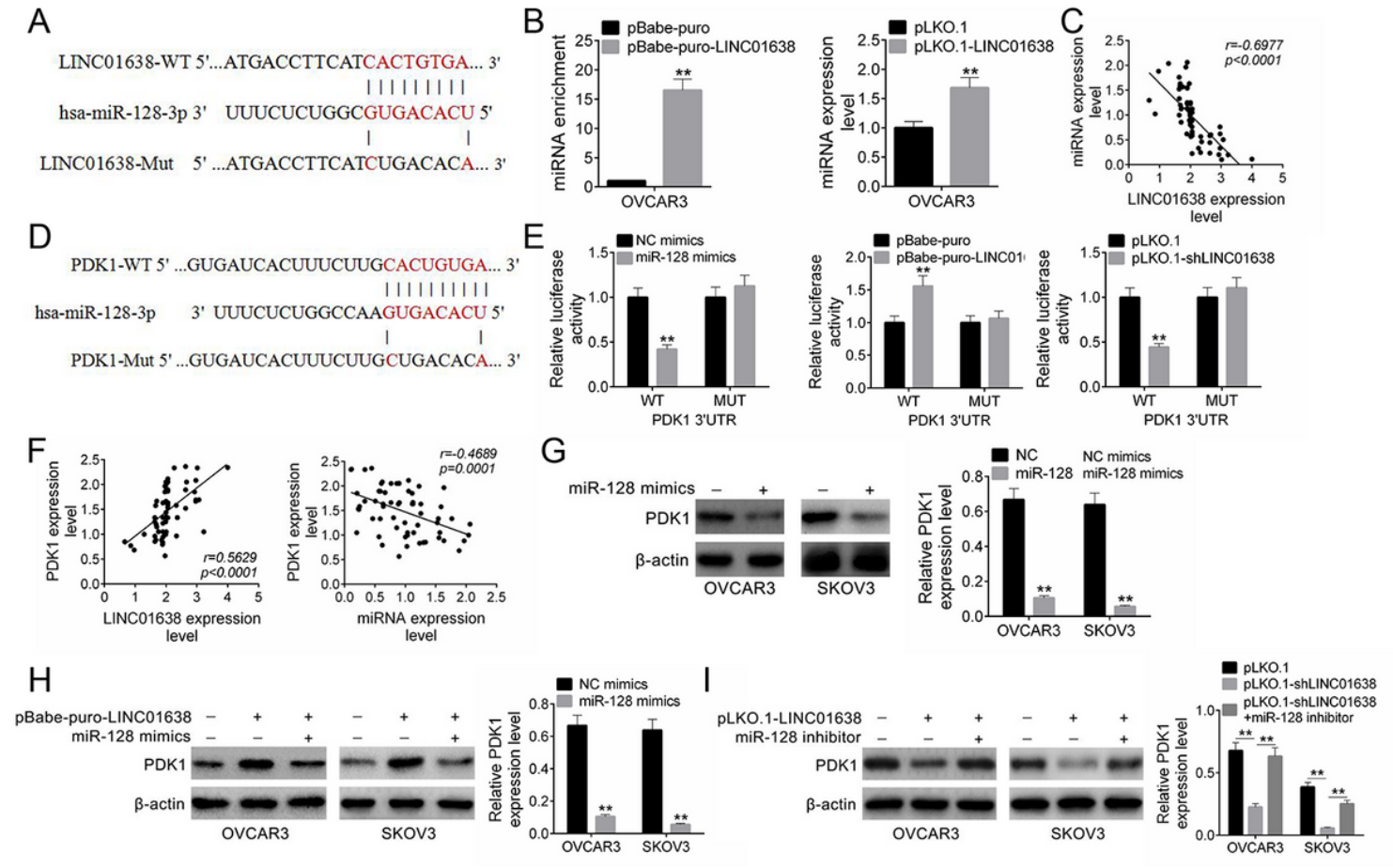
**Knockdown LINC01638 inhibited proliferation and survival of OC cells.** OC cell lines SKOV3 and OVCAR3 were transfected with pLKO.1-shLINC01638/pLKO.1 plasmids. (A) The transfection efficiency was detected using qRT-PCR. (B) Cell proliferation was detected using CCK-8 assay. (C) Cisplatin IC50 value was analyzed and calculated using MTT assay and GraphPad Prism software 6.0, respectively.. (D) Cell colony formation was detected using colony formation assay. (E) Cell apoptosis was detected using flow cytometry. (F) Cell cycle was detected using flow cytometry. ***P*<0.01 vs pLKO.1 group.




## Figure 3

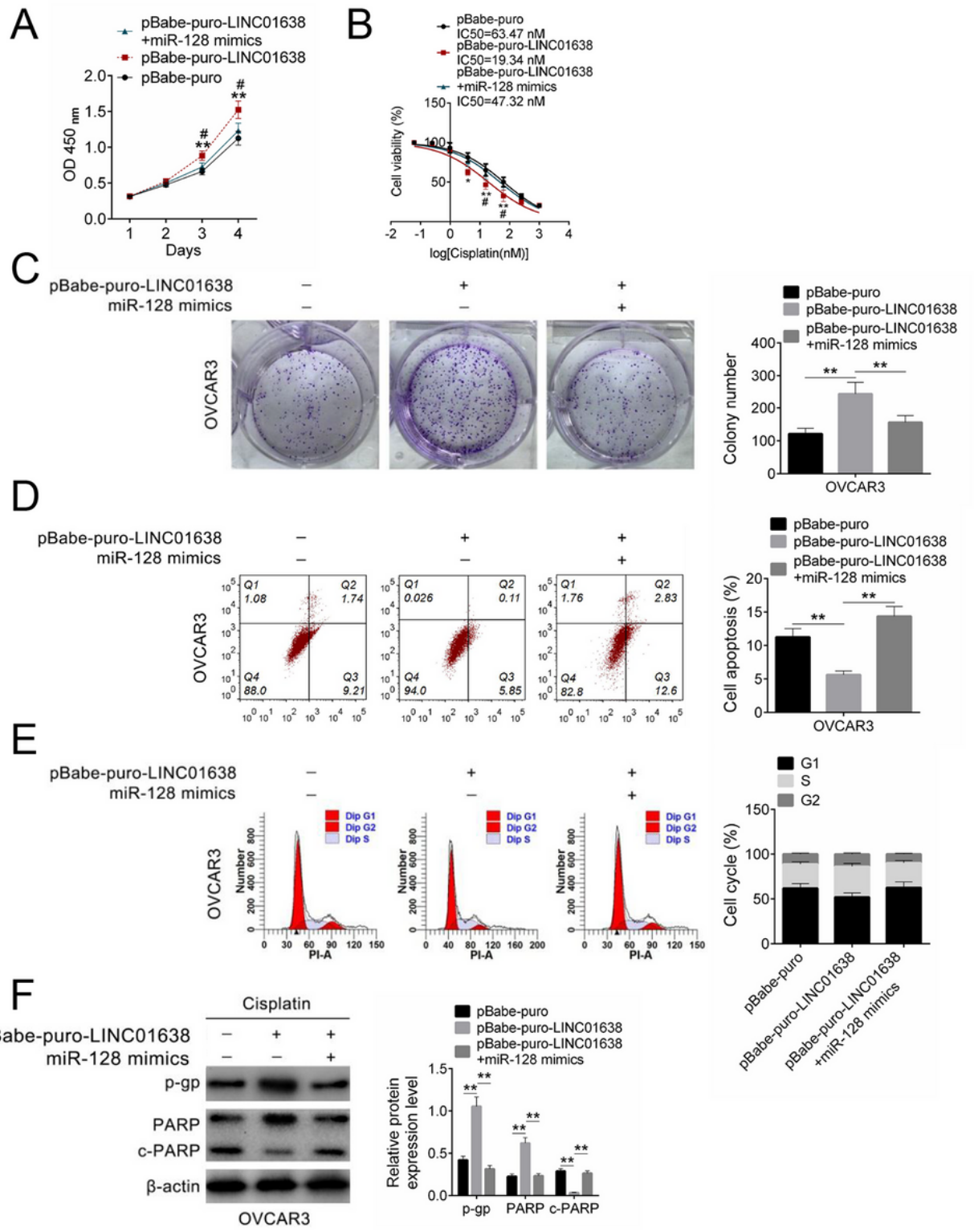
**LINC01638 caused cisplatin resistance in OC *in vivo* and *in vitro*.** OC cell lines SKOV3 and OVCAR3 were transfected pLKO.1-shLINC01638/pLKO.1 or pBabe-puro-LINC01638/pBabe-puro and treated with cisplatin/DMSO. Nude mice were injected with OVCAR3 (transfected with pLKO.1-shLINC01638/pLKO.1) and/or cisplatin. (A-B) The protein expressions of p-gp, PARP, c-PARP and β-actin were detected using western blot. (C) The tumor size of OC mice model was assesed after the mice were killed. (D) The tumor

volume of OC mice model was assesed weekly. (E) The tumor weight of OC mice model was assesed after the mice were killed. (F) The tumor proliferation and apoptosis were determined using Ki-67 assay and TUNEL, respectively. *$P$<0.05, **$P$<0.01 vs pLKO.1 group or pBabe-puro group.




## Figure 4

**LINC01638 directly targeted to miR-128-3p/PDK1.** (A) The binding sited of LINC01638 on miR-128-3p were predicted by miRDB. (B) The enrichment of miR-128-3p was detected using qRT-PCR after RNA pull down experiment. (C) The relationship between LINC01638 expression and miR-128-3p expression was analyzed using Pearson's χ2 test. (D) The binding sited of PDK1 on miR-128-3p were predicted by Targetscan. (E) The binding of PDK1 and miR-128-3p/LINC01638 was validated by luciferase reporter analysis.(F) The relationship among LINC01638, miR-128-3p and PDK1 expressions was analyzed using Pearson's χ2 test. (G-I) The protein expression of PDK1 was detected using western blot. **P < 0.01 vs pLKO.1 group, pBabe-puro group or NC mimics group.




## Figure 5

**Rescue experiment proved that LINC01638 affected OC cell proliferation, survival and cisplatin resistance through regulating miR-128-3p.** (A) Cell proliferation was detected using CCK-8 assay. (B) Cisplatin IC50 value was analyzed and calculated using MTT assay and GraphPad Prism software 6.0, respectively. (C) Cell colony formation was detected using colony formation assay. (D) Cell apoptosis was detected using

flow cytometry. (E) Cell cycle was detected using flow cytometry. (F) The protein expressions of p-gp, PARP, c-PARP and β-actin were detected using western blot. ** $P<0.01$ vs pBabe-puro group.